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Report

Sex Peptide Receptor and Neuronal TOR/S6K Signaling Modulate Nutrient Balancing in *Drosophila*

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Summary

Animals often decide between alternative actions according to their current needs, and hence the value they assign to each of the competing options [1–4]. This process is of special relevance during nutrient balancing, in which animals choose between different food sources according to their current nutritional state [5–7]. How such value-based decision making is implemented at the molecular and neuronal level in the brain is not well understood. Here we describe *Drosophila melanogaster* food choice as a genetically tractable model to study value-based decision making in the context of nutrient balancing. When faced with a choice between yeast and an alternative food source, flies deprived of protein prefer the yeast. We show here that mating status is a critical modulator of this decision-making process in females and that it relies on the action of the sex peptide receptor in internal *ppk*$^+$ sensory neurons. Neuronal TOR/S6K function is another critical input to this decision, possibly signaling the fly's current nutritional status. We propose that the brain uses these internal states to assign value to external sensory information from potential food sources, thereby guiding food choice and ensuring nutrient homeostasis.

Results

Drosophila melanogaster Makes Value-Based Feeding Decisions during Nutrient Balancing

We adapted a classic two-choice feeding preference test [8] to allow adult flies to choose between an inherently attractive but protein-free food source and one containing yeast as a protein source. In our assay, these two food sources were provided in solution with agar on a plate, mixed with either red or blue dye (Figure 1A). The dyes that we used are visible through the fly's abdomen and thereby afford a simple measure of its feeding choice. Flies were left to feed on the two food sources for 2–3 hr. The abdomen of each individual fly was then inspected to determine its preferred food source, from which a population yeast preference index was calculated (Figure 1B).

In this food choice assay, flies that had previously fed ad libitum on standard fly medium ingested little or none of the yeast-containing food (Figure 1C). Thus, for well-nourished flies, yeast does not appear to have a high value. To test whether flies adjust their food preference according to their metabolic needs, we selectively deprived them of yeast for several days prior to the choice assay. After 3 days of yeast deprivation, mated females now strongly preferred the yeast (Figures 1C and 1D; see also Table S1 available online). Males, in contrast, required much longer periods of yeast deprivation to show a similar switch in food choice (Figure 1D; Table S1). The switch in food preference depended on nutritional status, not age, because flies did not prefer yeast when maintained for similar periods on complete medium (Figure 1E). These sexually dimorphic changes in food choice were dependent on the presence of yeast in the feeding assays and were similar across a wide range of wild-type lines, indicating that this switch is a general adaptive phenomenon (Figure S1). Thus, flies adjust their food choice according to nutritional needs, with males and females switching food preferences with distinct dynamics.

If flies continually adjust their food choice according to nutritional status, we would expect the yeast preference to disappear as they recover from yeast deprivation. To test this prediction, we first deprived females of yeast for 3 days and then returned them to a complete medium for periods of up to 3 days before assaying their food preference. Females lost their preferences for yeast after 2 or more days on the complete medium (Figure 2A; Table S2). Males lost their yeast preference even more rapidly, despite the longer period of yeast deprivation required to achieve a similar level of yeast preference (Figure 2B; Table S2). All wild-type strains that we tested showed a similar recovery from yeast deprivation (Figure S2).

Taken together, these data demonstrate that flies of either sex continuously adjust their foraging behavior to compensate for the lack of a specific macronutrient, as has been described for other insects previously [5]. In the context of value-based decision-making theory [1–4], the value of yeast is adjusted according to nutritional status, thus making *Drosophila* food choice a powerful paradigm for studying the neural and molecular mechanisms of value-based decision making. As a first step in identifying mechanisms controlling the valuation of food, we next considered two candidate neural molecular pathways predicted to impact the valuation mechanism for yeast as a food source: the sex peptide receptor (SPR) and the TOR/S6K pathway.

Mating Status Affects Yeast Preference via SPR Action in *ppk*$^+$ Neurons

Mating induces drastic changes in the physiology and behavior of *Drosophila* females, including an increase in both egg laying and food intake [9, 10]. Yeast is a critical protein source for egg production [11], and so we wondered whether mating affects food preference as well as food intake. Indeed, in contrast to mated females, virgin females did not prefer yeast even after 3 days of yeast deprivation (Figure 3A; Figure S3A). Evidently, yeast becomes a more valuable food source for females after mating, presumably because dietary protein is a limiting factor in egg production [11].

Many of the behavioral changes that occur upon mating are triggered by signaling through SPR [12]. To test whether SPR signaling also regulates food choice, we tested mated *SPR* mutant females for their response to protein deprivation. Indeed, mated females lacking *SPR* behaved like virgins, in that they continued to avoid yeast even after 3 days of yeast

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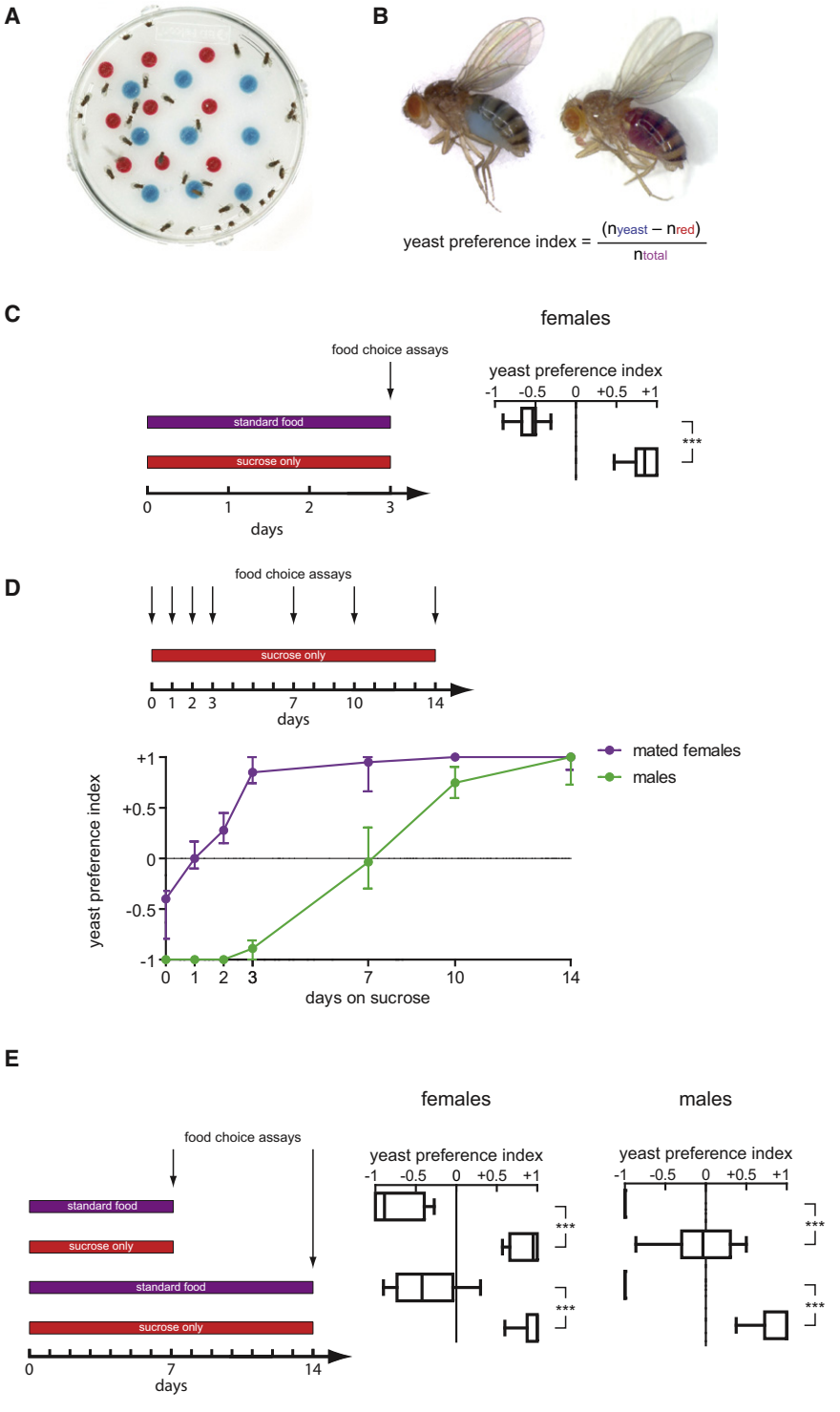

Figure 1. Feeding Decisions in *Drosophila* Nutrient Balancing

(A) Flies in the food choice setup (yeast is blue).

(B) Food choice was revealed by the color of the fly's abdomen after 2 hr of feeding, and a yeast preference index was calculated for the population. n_{yeast} is the number of flies with blue abdomens, n_{red} is the number of flies with red abdomens, and n_{total} is the number of flies that fed (blue, red, or red and blue abdomen).

(C) Protocol and yeast preference indices for well-fed and yeast-deprived females. Arrow indicates the time point of the assay. Box plots in (C) and (E) display the median, interquartile range, and 5-95 percentile whiskers. ***p < 0.001, Mann-Whitney test.

(D) Food choice assays after various periods of yeast deprivation (arrows). Data are median with interquartile range.

(E) Feeding choice reflects yeast deprivation, not age. ***p < 0.001, Mann-Whitney test.

deprivation (Figure 3B). SPR is not required for the induction of yeast feeding itself, because it is not required for males to switch their food preference after 10 days of yeast deprivation (Figure 3C). Thus, SPR is specifically required in females to modulate their food choice as a consequence of mating.

Other behavioral changes triggered by mating require SPR function in a set of *ppk*$^+$ reproductive tract sensory neurons [13, 14]. These same neurons are also critical for the switch in food choice behavior, because normal feeding preferences were largely restored in *SPR* mutant females by re-expressing *SPR* specifically in the *ppk*$^+$ neurons (Figure 3D).

The principle ligand for SPR in the regulation of female postmating behavior is the sex peptide (SP), a small protein present in the male's seminal fluid [9, 12]. Other SPR ligands have also been identified, but their functional roles remain unclear [12, 15, 16]. To test whether SP or some other SPR ligand regulates nutritional decision making, we examined the food choices of females flies mated with *SP* mutant males. These females showed an intermediate switch in their food preference, in that they showed a greater preference for yeast than virgin females did, but it was not as pronounced as that of females mated to control males (Figure 3E; Figure S3B). We conclude that SP indeed contributes to female nutritional decision making, but that other SPR ligands are also involved.

Mating and SPR activation also stimulate egg deposition [9, 12], and so the increased preference of mated females for yeast could be a secondary consequence of the faster depletion of protein reserves. To test this, we examined the food preferences of females carrying the dominant *ovoD1* mutation, which leads to female sterility and an early arrest in egg production [17]. Despite the block in egg production, these *ovoD1* females still showed a normal switch to yeast preference after 3 days of protein deprivation (Figure 3F). The rapid change in nutritional selection observed in mated females therefore cannot be due to an accelerated loss of protein as a consequence of egg production but is an independent and direct effect of SPR signaling in the nervous system. This result is in agreement with studies in other insects, which have suggested a non-demand-mediated mechanism in which

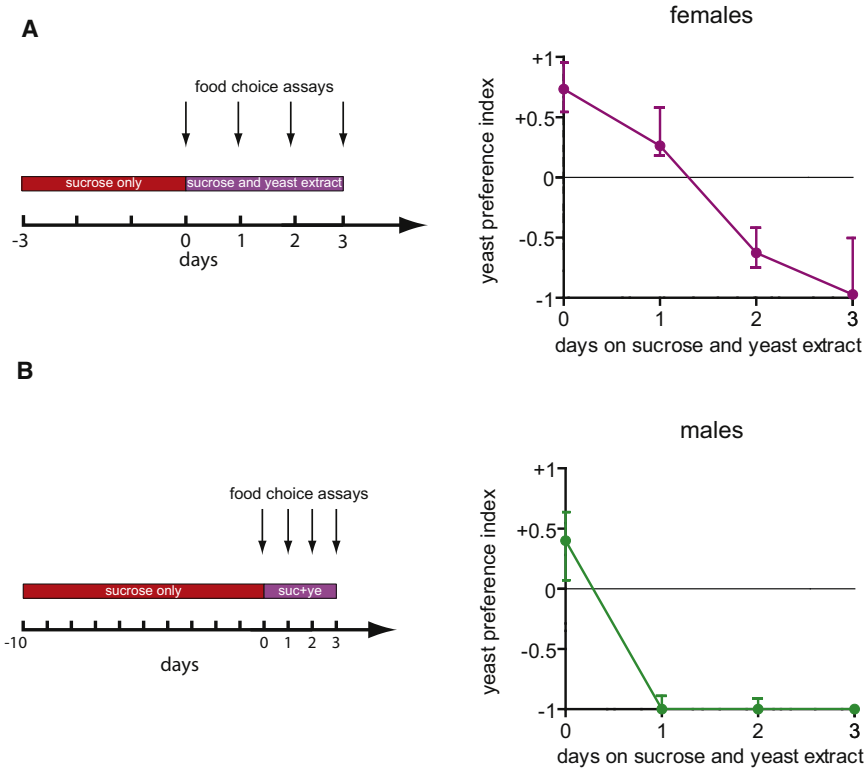

Figure 2. Food Choices Reflect Current Nutritional State

Food choices of females deprived of yeast for 3 days (A) and males deprived of yeast for 10 days (B) following recovery from yeast deprivation by feeding for various periods on sucrose (suc) and yeast extract (ye). Data are median with interquartile range.

protein intake is not regulated by the requirement for egg production [18].

The Neuronal TOR/S6K Nutrient-Sensing Pathway Modulates Yeast Preference

A second candidate signaling pathway likely to impact nutritional decision making is the highly conserved TOR (target of rapamycin)/S6K (RPS6-p70-protein kinase) pathway [19]. To match feeding decisions to current nutritional needs, the nervous system must directly or indirectly detect the fly's nutritional status, and the TOR/S6K pathway is one of the best-understood pathways involved in nutrient sensing. TOR is a key protein kinase integrating growth factor signaling, extracellular amino acid availability, and intracellular ATP levels to coordinate cell growth via protein synthesis, nutrient transport, and autophagy. Low amino acid or ATP levels lead to the activation of the tuberous sclerosis complex proteins Tsc1 and Tsc2 [20, 21], which in turn inhibit Rheb (Ras homolog enriched in brain), a small GTPase required for TOR activation [20, 22]. In rodents, hypothalamic TOR signaling has been shown to regulate bulk food intake [23], as has neuronal S6K signaling in *Drosophila* [24]. However, a role for TOR/S6K signaling in food choice has not been demonstrated in any species.

We hypothesized that yeast deprivation should lead to a decrease in the availability of free amino acids in the fly, which could be sensed through inhibition of TOR/S6K signaling in the nervous system. If so, inhibition of neuronal TOR/S6K signaling by overexpression of *Tsc1* and *Tsc2* [21] should mimic the effects of amino acid deprivation and hence lead to increased yeast feeding in our food choice assays. Consistent with this hypothesis, neuronal overexpression of *Tsc1* and *Tsc2* induced yeast feeding specifically in males deprived of yeast for 3 days, whereas control males with normal *Tsc1* and *Tsc2* levels avoided it (Figure 4A). A similar effect was seen upon inhibition of neuronal TOR/S6K signaling with a dominant-negative TOR protein (TOR^{TED}; Figure S4). However, TOR^{TED}, but not *Tsc1/Tsc2* overexpression, also disrupted the feeding choices of females in these assays, and these females appeared severely bloated and had difficulty walking. Thus, TOR inhibition through *Tsc1/Tsc2* overexpression leads to a specific defect in food choice, whereas inhibition via TOR^{TED} may lead to a more general disruption of neuronal function.

The TOR/S6K pathway can be artificially activated through either overexpression of *Rheb* [22] or expression of a constitutively activated form of the TOR effector S6K [25]. Activating TOR/S6K signaling by either of these means is predicted to signal abundant amino acid reserves and hence to suppress the switch to yeast feeding. Surprisingly, however, we found that *Rheb* overexpression (Figure 4B) and expression of dominant-active S6K ($S6K^{T398E}$; Figure 4C) both enhanced yeast feeding in our assays. Thus, both inhibition and activation of neuronal TOR/S6K signaling stimulate yeast feeding, suggesting a more complex role for neuronal TOR/S6K signaling in nutrient balancing. One possible explanation for these results is that TOR/S6K signaling reacts not only to the available levels of amino acids but also to carbohydrate levels [19, 26, 27] and so might act in distinct neuronal populations to signal distinct nutrient requirements. Regardless of its precise role in feeding behavior, the TOR/S6K pathway is unlikely to be activated through insulin-like receptor (InR) signaling, because neuronal manipulations of the InR downstream pathway did not affect food choices in our assays (Figure 4D).

Discussion

We have shown here that *Drosophila melanogaster* makes adaptive food choices according to current nutritional requirements, sex, and mating status. *Drosophila* food choice thus provides a tractable and ecologically relevant model to elucidate the neural and genetic mechanisms of value-based decision making and nutrient balancing. We have defined two key factors in *Drosophila* nutritional decision making. First, SPR signaling in ppk^{+} neurons induces a strong preference for yeast feeding in mated females, concomitant with but not dependent upon increased egg production. Second, neuronal TOR/S6K signaling modulates food choices, possibly through its complex and still poorly understood role in sensing internal reserves of multiple nutrients. Further support for these

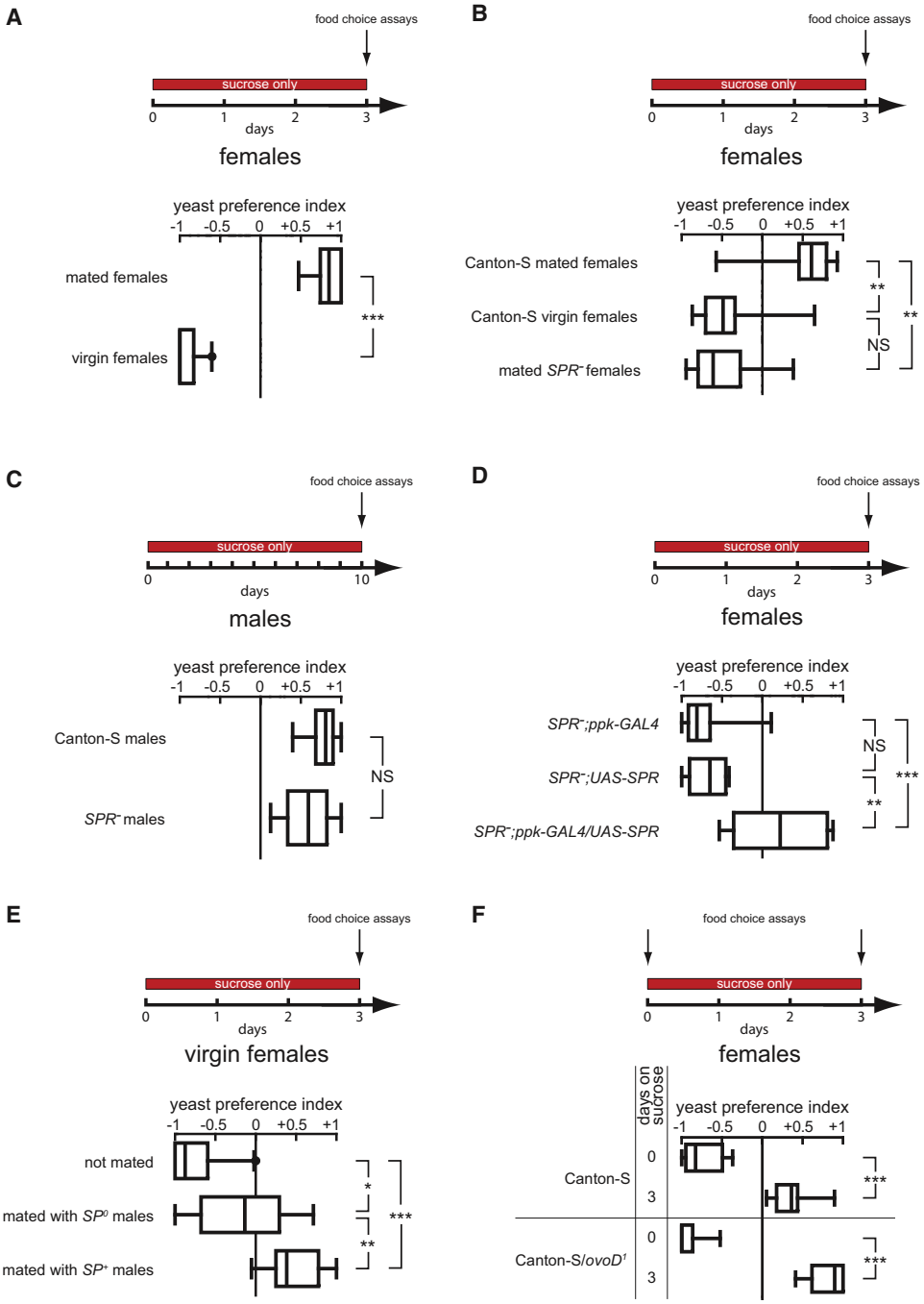

Figure 3. Mating Status Affects Female Feeding Decisions via SPR Signaling in *ppk*$^+$ Neurons

(A) Feeding decisions of mated and virgin females after 3 days of yeast deprivation.
(B and C) Food choices of wild-type and *SPR* mutant females (B) and males (C).
(D) *SPR* rescue in *ppk*$^+$ neurons.
(E) Food choices of unmated females and females mated with *SP*^{0} males (lacking SP) and control males (*SP*$^+$).
(F) Food choices of females carrying the dominant *ovoD*^{1} allele.
Box plots display the median, interquartile range, and 5-95 percentile whiskers, with data beyond these whiskers shown as points. Significance was tested by Mann-Whitney test in (A), (C), and (F) and by Kruskal-Wallis test followed by Dunn's multiple comparison test in (B), (D), and (E). NS, not significant ($p > 0.5$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

conclusions comes from an independent study, reported in this issue of *Current Biology* [28], which also defines a critical role for both mating and *dS6K* in nutrient balancing in *Drosophila*.

The TOR/S6K pathway may have an analogous role in regulating the host-seeking behavior of the malaria mosquito *Anopheles gambiae*. Ingestion of amino acid-rich diets suppresses mosquito biting behavior in a manner similar to that described here for *Drosophila* yeast feeding and is furthermore correlated with changes in neuronal TOR signaling [29]. We propose that the brain uses internal states set by mating

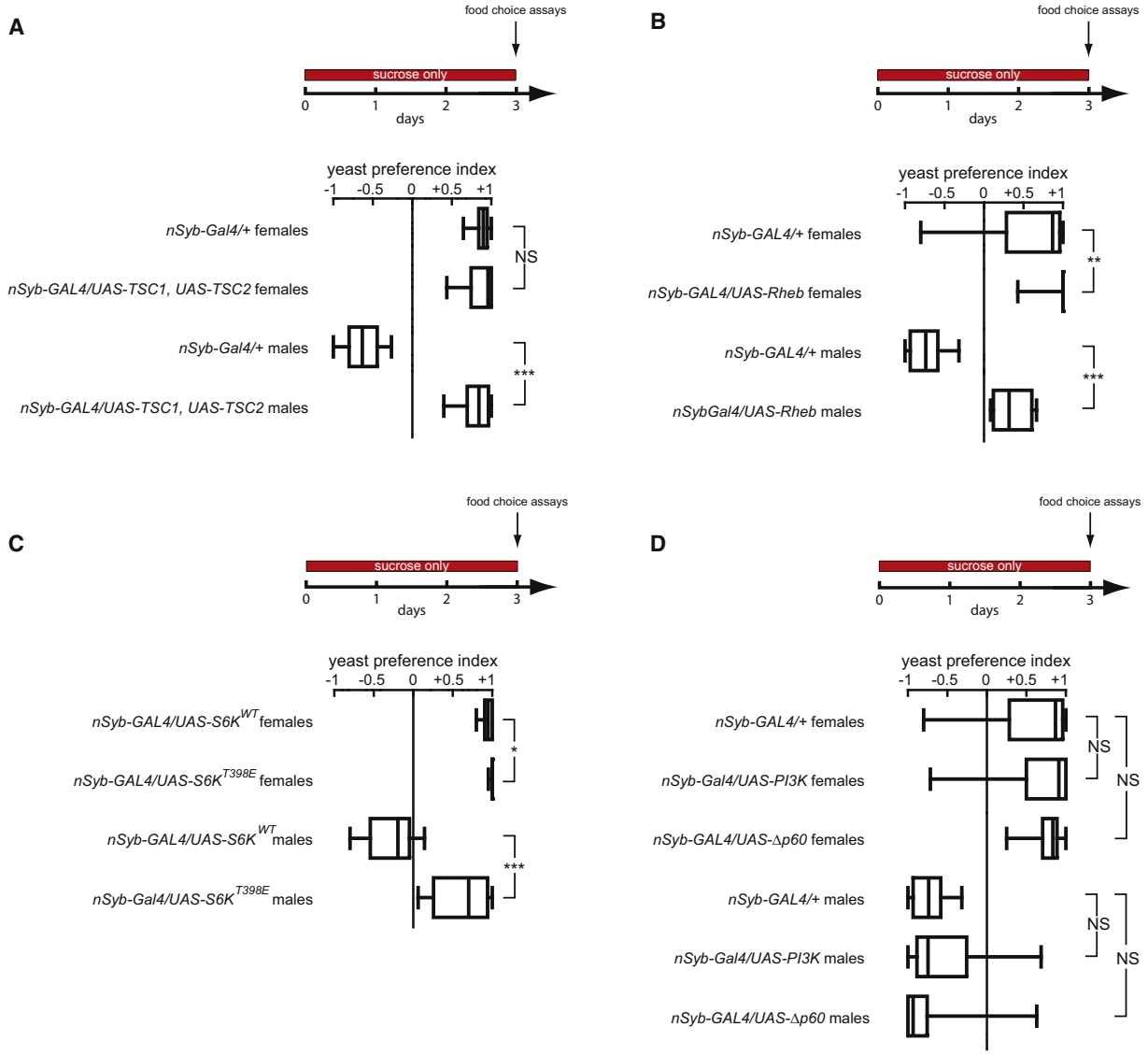

Figure 4. Neuronal TOR/S6K Signaling Modulates Food Choices

(A–C) Effect of overexpressing the negative TOR regulators TSC1 and TSC2 (A), the positive TOR regulator Rheb (B), and a constitutively active form of S6K (S6K^{T398E}; C) throughout the nervous system. In each case, an increase in yeast preference is observed in males, and sometimes in females.

(D) Food choices are not altered by activation (overexpression of *PI3K*) or inhibition (overexpression of the dominant-negative PI3K subunit Δp60) of the insulin-like receptor (InR) pathway.

Box plots display the median, interquartile range, and 5-95 percentile whiskers, with data beyond these whiskers shown as points. Significance was tested by Mann-Whitney test in (A)–(C) and by Kruskal-Wallis test followed by Dunn's multiple comparison test in (D). NS, not significant ($p > 0.5$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

and nutritional status to assign value to external sensory information from potential food sources, which is then used to guide food choice. The behavioral paradigm that we have established for *Drosophila* provides an excellent opportunity to apply powerful genetic methods to further investigate how neural systems adjust an animal's foraging behavior according to its metabolic needs.

Experimental Procedures

Drosophila Stocks and Genetics

Unless otherwise stated, control flies were from our standard background for behavioral experiments, obtained by crossing *elav-GAL4* virgins to w^- males. *SP* mutant males were generated by crossing *SP^0/TM3,Sb* to *Δ^{130}/TM3,Sb*; *SP* control males were generated by crossing *SP$^+$/TM3,Sb* to *Δ^{130}/TM3,Sb.* Females lacking the ability to produce eggs were generated by crossing Canton-S females to *ovoD^1* males. Virgins were obtained by heat shocking third-instar larvae from crosses of lines bearing a *hs-hid* construct on the Y chromosome. In the InR/TOR/S6K pathway experiments, *nSyb-Gal4* crossed to w^- was used as control background. For detailed genotypes of all lines used, see Tables S3–S5.

Behavioral Assays

Fifteen to thirty 1- to 4-day-old adult flies were collected and maintained on fresh fly food for 3 days. For mated females, three to six males were added. To ensure that the flies were well fed, we transferred them to fresh food at least every second day. Flies were either assayed on the third day or transferred to tubes containing fresh fly food, 5 ml of 100 mM sucrose (Fluka 84097), or 5 ml of 100 mM sucrose and 2% yeast extract (Sigma Y1625). These flies were then transferred to fresh media every 3–4 days.

The behavioral assay for nutrient selection was an adaptation of the classic two-choice feeding preference test [8]. Aliquoted and frozen

0.75% agarose solution containing 20 mM sucrose mixed with 0.125 mg/ml of the blue dye indigo carmine (Sigma I8130) or 0.75% agarose solution containing 5% yeast (SAF instant yeast) mixed with 0.5 mg/ml of the red dye amaranth (Sigma A1016) was melted in a water bath at 70°C, and nine 10 μl spots of each solution were applied on a Petri dish with a tight-fit lid (Falcon 35-1006) in a checkerboard pattern (Figure 1A). When given the choice between the amaranth (red)- and indigo (blue)-containing media, flies preferred the red medium even in the absence of sucrose. The yeast concentration was calibrated to obtain a clear yeast preference after 3 days of yeast deprivation in females while not having all flies eat exclusively from the yeast (Figures S1A and S1B).

Flies were mildly anesthetized with CO_2, transferred to the prepared Petri dish, left to feed for 2–3 hr in a humidified temperature-controlled incubator at 25°C in the dark, and subsequently frozen. The abdomen of each fly was visually inspected, and the fly was scored as having eaten the yeast-free medium (red abdomen), yeast (blue abdomen), or both (red and blue or purple abdomen) (Figure 1B). The yeast preference index for the whole population in the assay was calculated as follows: (number of blue flies − number of red flies)/(number of red flies + number of red and blue flies + number of blue flies). All assays were performed on at least two different days. Unless otherwise stated, the number of assays is at least eight. Data were analyzed with GraphPad Prism 5.

Supplemental Information

Supplemental Information includes five tables and four figures and can be found with this article online at doi:10.1016/j.cub.2010.03.061.

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